SUPPLEMENTAL MATERIAL:

Derepression of the *Bacillus subtilis* PerR peroxide stress response leads to iron deficiency

Melinda J. Faulkner, Zhen Ma, Mayuree Fuangthong, and John D. Helmann

Department of Microbiology
Cornell University,
Ithaca, NY

Table S1: Comparison of CPIII and the unidentified tetrapyrrole (*TP*)

Properties	CPIII	TP
λ_{max} (Absorbance)	392 nm	404 nm
λ_{max} in 10 mM HCl)	400 nm	400 nm
$\lambda_{exitation}$	402 nm	402 nm
$\lambda_{emission}$	581 nm	581 nm
m/z in ESI-MS	655	689 and 889

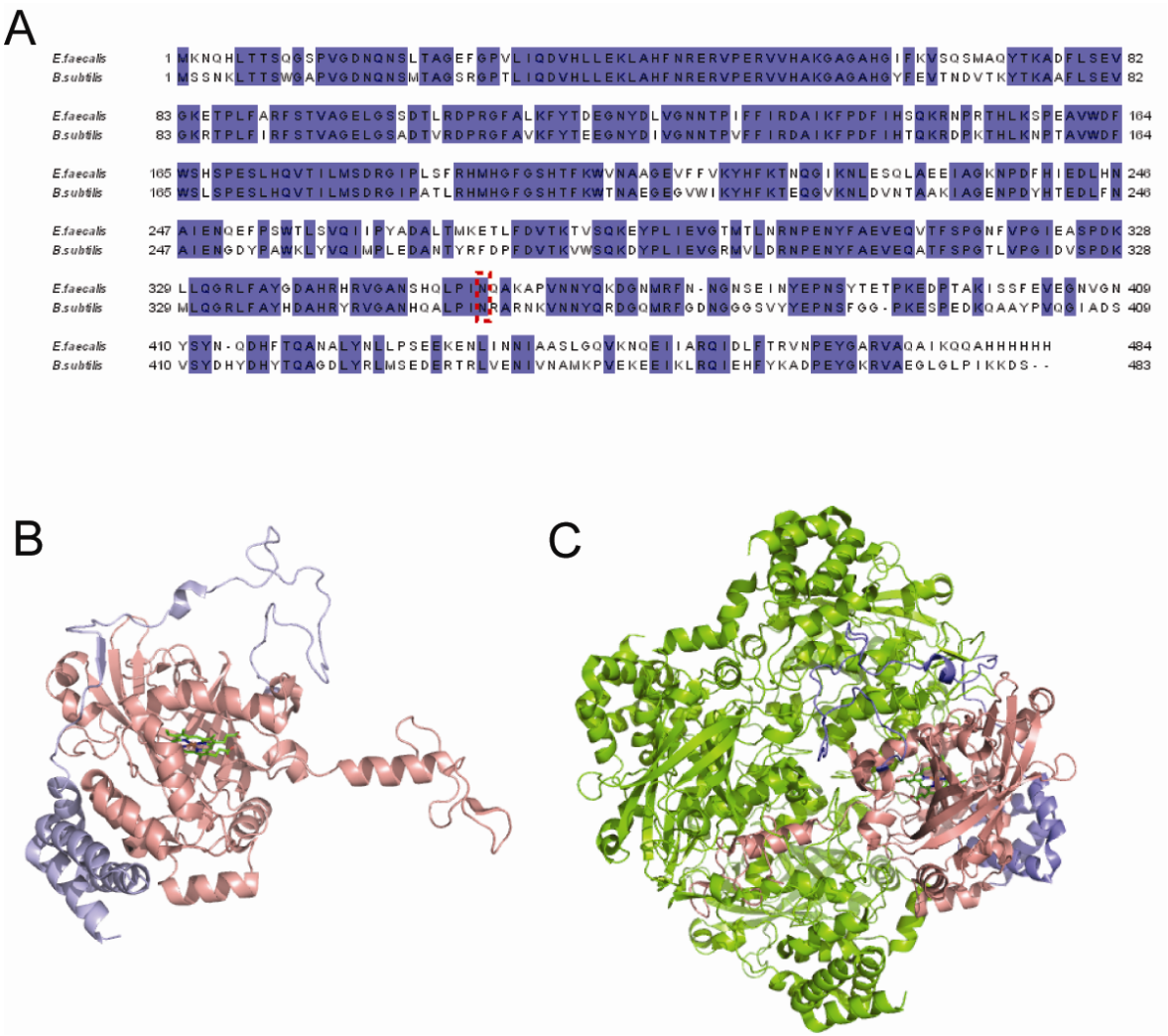

Figure S1.: *B. sutbilis* KatA355stop still contains the whole heme binding pocket. (A) Sequence alignment of *B. subtilis* KatA and *E. faecalis* catalase. Red box indicates residue 355. (B) Structure of *E. faecalis* catalase (PDB ID: 1SI8) monomer with the C-terminal tail after the corresponding residue 355 in blue. (C) Structure of *E. faecalis* catalase tetramer. One subunit is highlighted using the same color scheme as (B).

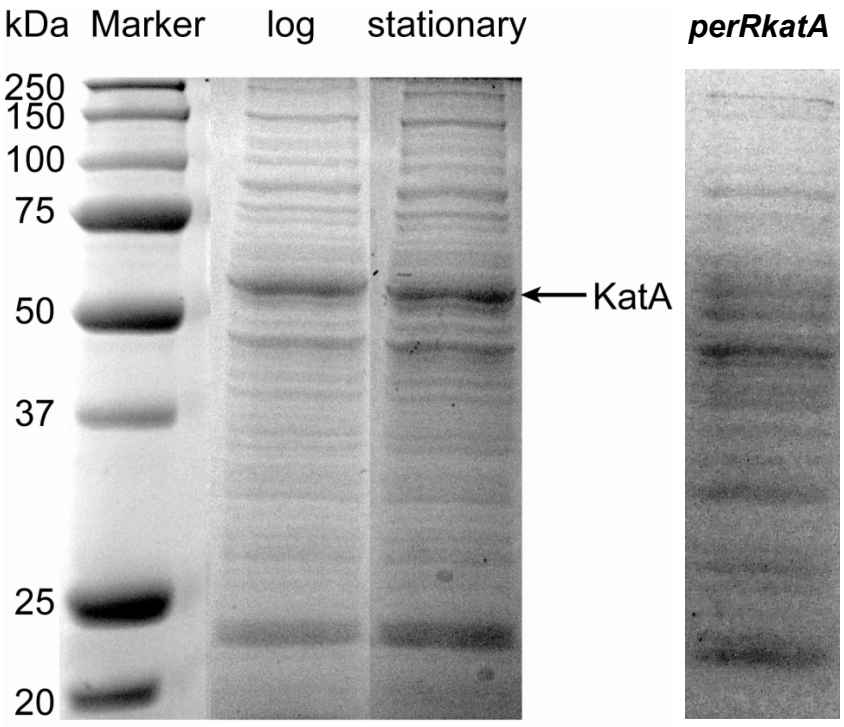

Figure S2: Visualization of KatA in *B. subtilis* cell (HB14009) extracts by coomassie blue stained SDS-PAGE gel. Lysates from mid-log and stationary phase cells containing 13.2 µg and 15.6 µg total protein were loaded as indicated. The amount of KatA protein was quantified using lysozyme as standard, which was added at a concentration of 0.2 µg/µL during cell lysis. Multiple samples with different amount of proteins were loaded for better quantification. Lysates from *perRkatA* containing 28.0 µg total protein (from a separate gel) areshown on the right as a control.

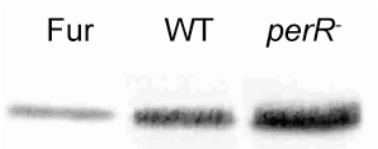

Figure S3: Western-blot of Fur protein with anti-Fur in WT and *perR*$^{-}$ *B. subtilis* cell extracts. Lane 1: Fur (1.4 ng) purified from *E. coli*; Lane 2: WT extracts (6 µg total protein); Lane 3: *perR* extracts (6 µg total protein).

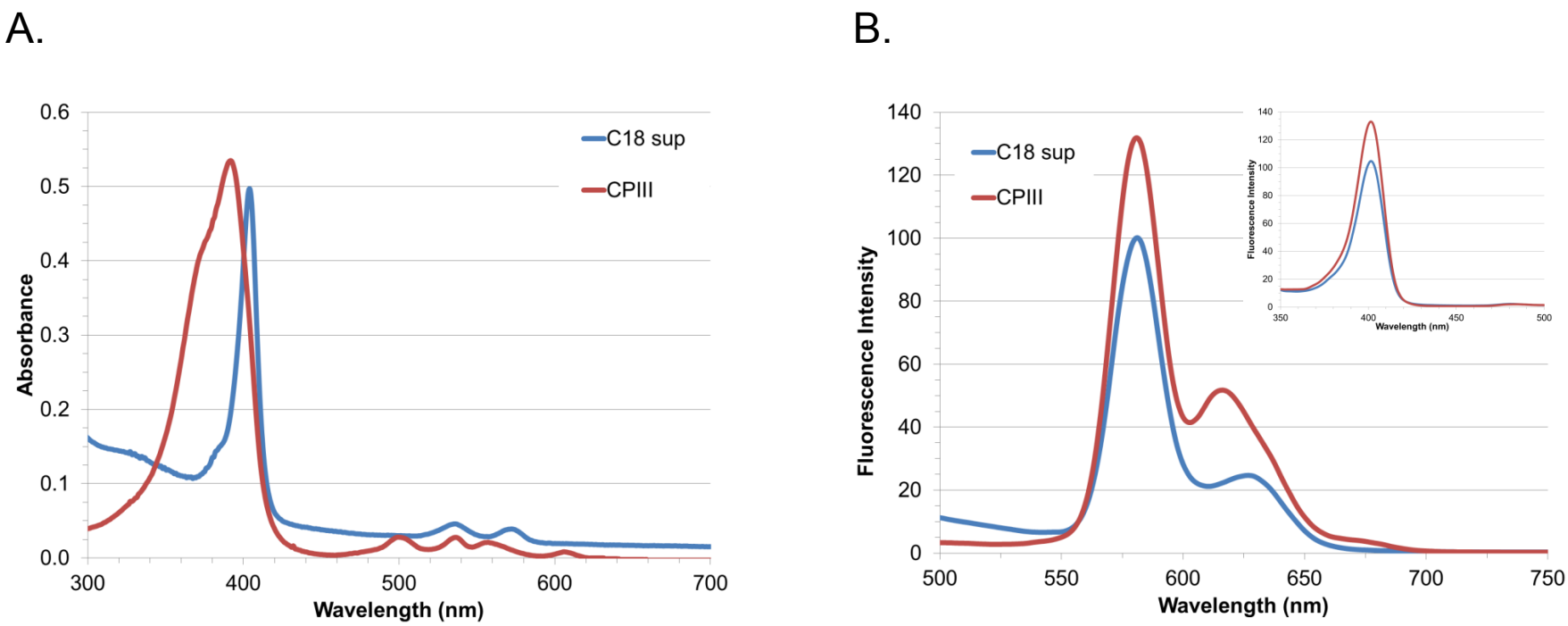

Figure S4. UV-visible and emission spectra of C18-purified supernatant. (A) UV-visible spectra of C18-purified supernatant and CPIII. (B) Emission spectra (λex = 402 nm) of C18-purified supernatant and CPIII. The excitation spectra (λem = 581 nm) are displayed in the inset.

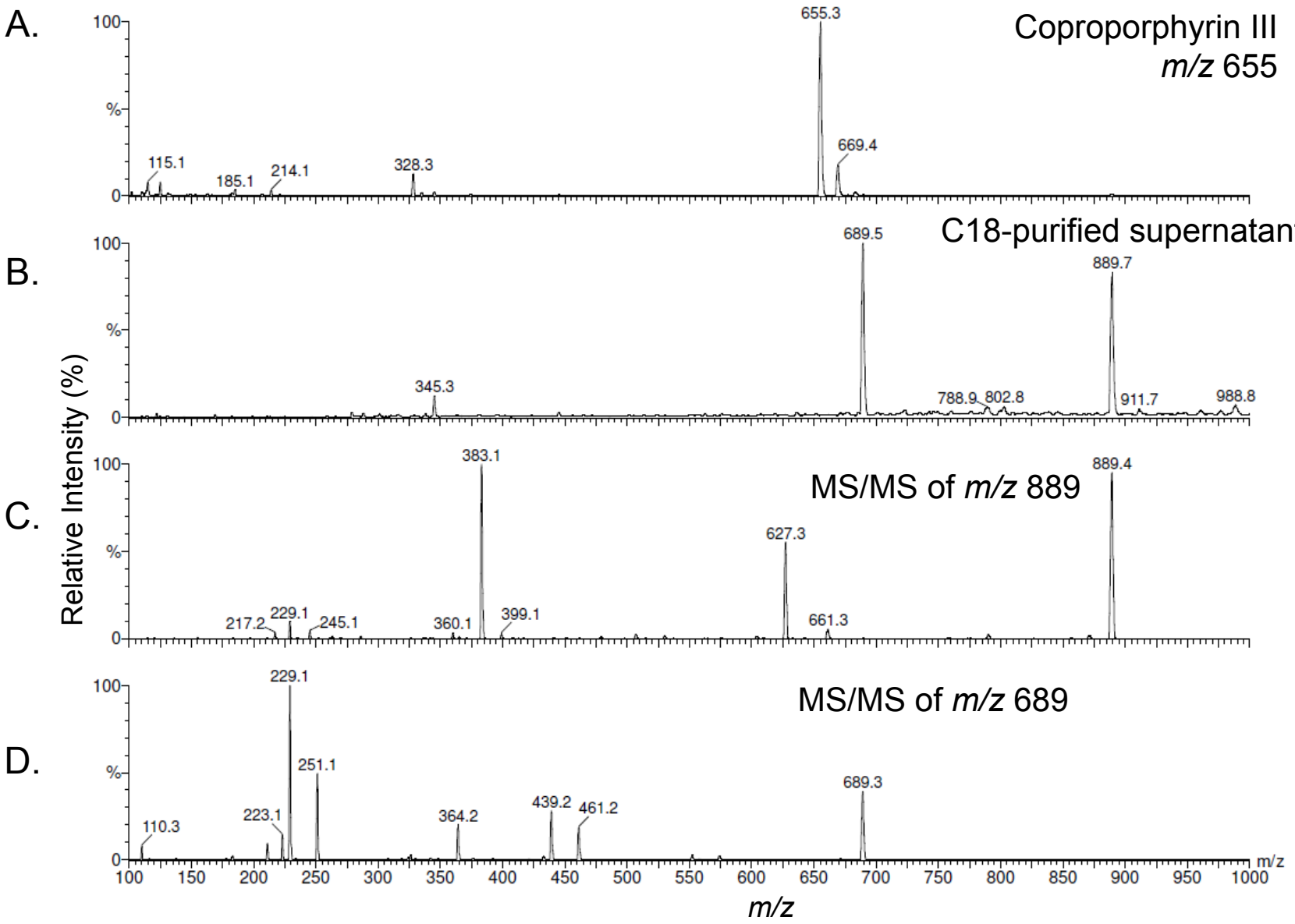

Figure S5. Electrospray ionization tandem mass spectrometry (ESI MS/MS) of the C18-purified supernatant. (A) ESI MS of coproporphyrin III. (B) ESI MS of C18-purified supernatant. (C) ESI MS/MS of m/z 889 in C18-purified supernatant at a collision energy of 27 eV. (D) ESI MS/MS of m/z 689 in C18-purified supernatant at a collision energy of 27 eV.

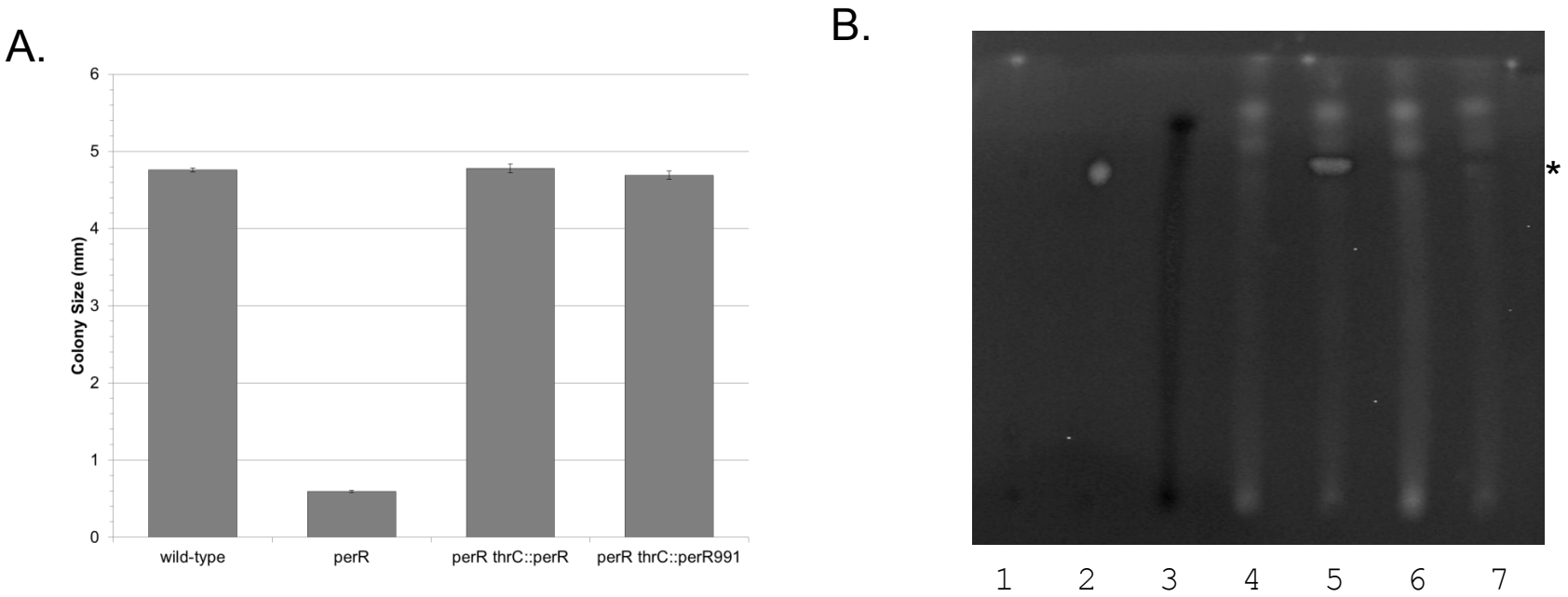

Figure S6. Characterization of the *perR991* strain. A. Complementation of a *perR* null allele with perR991 restores rapid growth (as monitored by colony size). B. Lack of accumulation of *TP* in the *perR*991 mutant strain. TLC of standards: lane 1, UPIII; 2, CPIII; 3, PPIX compared to the C18-purified supernatants from the indicated strains: lane 4, wild-type; 5, *perR*; 6, *perR*::spec *thrC*::*perR*; 7, *perR*::spec *thrC*:*perR*991.